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(54) Title: HUMAN TROPHOBlast AND CHORIOCARCINOMA INHIBITION

(57) Abstract

It was found that the depletion of HERV-PTN mRNA prevents human choriocarcinoma growth, invasion and angiogenesis. Growth, invasion and angiogenesis of a tumor containing human endogenous retrovirus-pleiotrophin (HERV-PTN) sequences may be inhibited by administration of a choriocarcinoma inhibiting amount of PTN-targeted ribozymes. A ribozyme of the formula: '5'-GTTGCAGGGCTGATGAGTCCGTTAGGACGAAATCTTACATC-3' was found to be particularly useful for this purpose.

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Title: Human Trophoblast and Choriocarcinoma Inhibition**Field of the Invention:**

This invention is related to a novel, tissue-specific promoter which provides a new means for evaluating progesterone-like products for use in treatment. The invention also provides means for preventing choriocarcinoma growth, invasion and angiogenesis by targeting the human endogenous retrovirus-pleiotrophin (HERV-PTN) mRNA transcript with PTN-targeted ribozymes.

Background of the Invention:

Retroviral elements are found in abundance throughout the human genome but only rarely have alterations of endogenous genes by retroviral insertions been described.

Formation of the trophoblast is one of the early differentiation events occurring in the developing embryo after it organizes into an inner and outer cell mass from the latter of which the trophoblast evolves during the first weeks. Interestingly, teratocarcinoma (PA-1) cells that stem from the undifferentiated embryoblast as well as adult tissues and tumor cells derived from different germ layers utilize the PTN promoter that is located in a region in common with the murine gene. In contrast, the phylogenetically novel HERV-PTN transcription unit is active in human trophoblast-derived normal and tumor cells and tissues, and the data suggests that activation of this promoter occurs early during the formation of the trophoblast.

Pleiotrophin (PTN) is a secreted, heparin-binding polypeptide growth factor with mitogenic and transforming effects on fibroblasts, and growth factor activity on epithelial and endothelial cells. Furthermore, PTN induces the release of proteolytic enzymes from endothelial cells and stimulates neurite outgrowth and tube formation by endothelial cells in vitro as well as angiogenesis in the rabbit corneal pocket assay. PTN gene expression is regulated in a time- and tissue-

specific manner during rodent development and PTN mRNA is found at high levels in the central nervous system during the perinatal period, is down-regulated thereafter, and is present at low levels in a few adult tissues. On the other hand, the PTN gene is up-regulated in several human tumor tissues and tumor cell lines. However, little is known about the regulatory elements in this gene.

Particles similar to Human endogenous retrovirus (HERV) were identified more than two decades ago in human oocytes, teratocarcinoma cells, mammary carcinoma tissues as well as in placenta, and retroviral transcripts were detected in a number of tissues and cell lines. These findings reflect the high number of HERV fragments found integrated throughout the human genome (several thousand copies). However, only one example of HERV germ line insertion that induces changes of the expression pattern of a functional human gene product has been reported to date: a C-type HERV was found integrated in reverse orientation into the 5' flanking region of human amylase genes and was shown to function as an enhancer that confers additional salivary gland expression of amylase.

Summary of the Invention:

It was found that the depletion of HERV-PTN mRNA prevents human choriocarcinoma growth, invasion and angiogenesis. Growth, invasion and angiogenesis of a tumor containing human endogenous retrovirus-pleiotrophin (HERV-PTN) sequences may be inhibited by administration of a choriocarcinoma inhibiting amount of PTN-targeted ribozymes. It has been found that the ribozyme of the sequence

'5-GTTGCAGGGCTGATGAGTCCGTTAGGACGAAATCTTACATC-3' is particularly useful for preventing human choriocarcinoma growth, invasion and angiogenesis. The sequence was found to be effective for use against trophoblastic tumor cells. The sequence may be administered in a pharmaceutically acceptable carrier or in a vector.

Detailed Description of the Invention:

The significance of HERV-PTN mRNA in a choriocarcinoma model was investigated by targeting this transcript with

ribozymes. It was found that the depletion of HERV-PTN mRNA prevents human choriocarcinoma growth, invasion and angiogenesis.

5 A human endogenous retrovirus (HERV) type C sequence was inserted in the human growth factor gene pleiotrophin (PTN) between the 5' untranslated and the coding region. This insert in the human genome expands the region relative to the murine gene. Investigations with promoter-reporter constructs show that the HERV insert in the human PTN gene generates an 10 additional promoter with trophoblast-specific activity. Due to this promoter function, fusion transcripts between HERV and the open reading frame of PTN (HERV-PTN) were detected in all 15 normal human trophoblast cell cultures as early as nine weeks after gestation (n=7) and in all term placenta tissues (n=5) but not in other normal adult tissues. Furthermore, only 20 trophoblast-derived choriocarcinoma cell lines expressed HERV-PTN mRNA whereas tumor cell lines derived from the embryoblast (teratocarcinoma) or from other lineages failed to do so.

25 To understand the mechanisms that regulate expression of the human PTN gene, 5' ends of PTN mRNA were isolated from different human tissues that express the PTN gene. Surprisingly, it was found that all placenta samples in contrast to brain samples, expressed PTN mRNA with 5' exons that are homologous to a human endogenous retrovirus (HERV) and are spliced onto the intact open reading frame (ORF) of PTN. Upon analysis of 30 human genomic DNA, it was possible to locate the insertion of an HERV fragment into the intron region upstream of the ORF of the human PTN gene expanding this region relative to the ancestral PTN gene shared with other non-primate species.

35 It is now demonstrated that germ line insertion of an HERV fragment generates a phylogenetically new promoter within the human PTN gene. This insertion confers trophoblast-specific expression of functional PTN gene products. Using methods disclosed herein, it was possible to evaluate the significance of this finding for the growth phenotype of human trophoblast-derived choriocarcinoma and discuss potential implications for the growth of the normal human trophoblast.

MATERIALS AND METHODS

5 **Cell Lines and Growth Assays.** Human choriocarcinoma (JEG-3, JAR), teratocarcinoma (PA-1), adrenal carcinoma (SW-13) cells were from ATCC and were grown in IMEM with 10% fetal calf serum (FCS; Life Technologies Inc., Gaithersburg, MD); human melanoma cells (1205LU; gift from Dr. M. Heerlyn, Wistar Institute, Philadelphia) in KSF/L15 media mixed at a ratio of 3:1 (Life Technologies Inc.) and supplemented with 5% FCS. Primary cells grown from chorionic villus samples obtained for 10 prenatal diagnostics were a gift of Dr. Simon (Georgetown University) and were kept in IMEM media with 20% FCS. To determine the proliferation rates of differently modified JEG-3 cells, 2x10⁴ cells were plated in triplicates into 6-well plates and the number of cells was counted at different time 15 intervals.

20 **Gene Structure Analysis.** Non-overlapping phagemid P1 clones (Genome Systems, St. Louis, MI) were used to complete the structural analysis of the 5' untranslated region of the human PTN gene reported earlier (Lai, S., Schulte, A. M., Wellstein, A. & Riegel, A. T. (1995) Gene **153**, 301-302 and Lai, S., Czubayko, F., Riegel, A. T. & Wellstein, A. (1992) Biochem. Biophys. Res. Commun. **187**, 1113-1122.). P1 clones containing the upstream untranslated exon U1 (P203) or the first exon of the ORF, O1 (P2258), were obtained by PCR screening with 25 specific primers. Long-range PCR (ExpandTM long template PCR; Boehringer Mannheim) with P1 clone 2258 (containing O1) or with human genomic DNA and subcloning and sequencing of inserts was used to compile the structure of the human PTN gene. The nucleic acid sequence of the inserted promoter region and of 30 HERV-derived exons UV3 and in part of UV2 were obtained from a genomic DNA fragment subcloned from a BamHI restriction library of P1 clone P2258 (containing O1) which was screened for positive clones with a UV3-specific probe. Furthermore, the 5' RACE PCR products of placenta cDNA were sequenced for 35 comparison and to complement data from the genomic cloning.

5' RACE PCR (=5' rapid amplification of complementary DNA ends polymerase chain reaction). Two 5' RACE cDNA libraries

generated from human placenta and human brain mRNA (Clontech, Palo Alto, CA) served to generate PCR fragments of the 5' ends of different PTN cDNAs. Nested primers derived from exon 01 were used as 3' primers in the PCR reaction and an antisense oligonucleotide to the anchor sequence was used as a 5' primer. 5 PCR fragments were subcloned into TA-cloning vectors (Invitrogen) and inserts were sequenced.

Mapping of the Transcription Start Site by Primer Extension. The AMV Reverse Transcriptase (RT) Primer Extension System (Promega, Madison, WI) was used with polyA⁺ RNA (9 µg) or total RNA (50 µg) as a template. RNA from JEG-3 and JAR choriocarcinoma cells (PTN-positive) or SW-13 cells (PTN-negative) was incubated with two UV3-specific, nested primers designed to hybridize to the sequence stretch between -11154 / -11170 and -11168 / -11184 respectively. After denaturation of the RNA for 30 minutes at 65°C, primer hybridization was run for one hour at 52°C followed by a one hour incubation at 42°C with AMV-RT. The samples were then heated for 10 minutes at 90°C in formamide loading buffer and analyzed on a 20 6% sequencing gel. Sequencing reactions with each of the primers were used to read the position of the extended product.

Transcriptional Activity. A 1.9 kb HindIII/BamHI (H,B) genomic fragment from P1 clone P2258 was used for these studies. This fragment starts upstream of the Alu region (- 25 12,534), contains the TATA box and transcription start site of the HERV-PTN fusion transcripts and ends in exon UV2 (- 10,640). The fragment was cloned in both orientations into the pXP-1 promoterless luciferase reporter gene vector by known methods (Czubayko, F., Riegel, A. T. & Wellstein, A. (1994) J Biol Chem. **269**, 21358-21363) and then used in transient 30 transfection assays in different cell lines. For this, cells were plated overnight at 60% to 70% confluence in 6 well plates and then transfected in Optimem (Life Technologies) with 1 µg of DNA per well using 7 µl of Lipofectamine (Life Technologies) for the JEG-3, JAR and SW-13 cells and 2.5 µl of 35 Transfectam (Pharmacia) for the 1205LU cells. After 5 hours, transfection media was replaced by fresh culture medium and the

5 cells were incubated for another 24 to 36 h. Thereafter cells were harvested, washed and lysed in 0.25 M Tris-HCl buffer, pH 7.8, freeze-thawed three times and 5 to 20 μ l of the lysate were mixed with 350 μ l of 0.1 M KPO₄, 15 mM MgCl₂, 5 mM ATP at pH 7.8 and assayed for luciferase activity using 1 mM D-luciferin as a substrate. The promoter activity is shown as fold induction relative to the parent vector pXP-1. A CMV-drive luciferase expression vector was used to control for transfection efficacy.

10 **Depletion of PTN mRNA using Ribozyme Targeting.** The PTN-targeted ribozyme Rz261 (See Czubayko, cited above) was expressed under the control of the tTA / heptameric operator binding site and a CMV minimal promoter (Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5547-5551). For this 15 purpose, the major portion of the luciferase gene and the SV40 polyadenylation site in the pUHC13-3 plasmid (Repaske, R., Steele, P. E., O'Neill, R. R., Rabson, A. B & Martin, M. A. (1985) *J Virol.* **54**, 764-772) were deleted by HindIII/HpaI cut and replaced with the Rz261/bovine growth hormone polyadenylation HindIII / PvuII fragment from the pRc/Rz261 expression 20 vector as taught by Czubayko, et al. The remaining luciferase start codon was replaced by a SalI/ClaI/ HindIII cassette to yield the construct pTET/Rz261. This ribozyme is designed to cleave PTN mRNA 3' of nucleotide 261 of the ORF (See Czubayko). 25 In JEG-3 cells the ribozyme expression vector (pTET/Rz261, 0.5 μ g), was co-transfected with the tTA expression vector (pUHG15-1 (See Gossen, cited above), 0.5 μ g) and pRc/CMV (0.1 μ g) to provide G-418 resistance. After selection 30 for stable integrants in the presence of 1 mg/ml of G-418, the cells were tested for PTN expression by Northern analysis.

35 **Northern Blot.** Total RNA from cell lines or tissues was isolated with the RNA STAT-60 method (Tel-Test Inc.; Friendswood, TX), separated and blotted as reported earlier (Fang, W. J., Hartmann, N., Chow, D., Riegel, A. T. & Wellstein, A. (1992) *J Biol. Chem.* **267**, 25889-25897). In addition, a human multiple tissue Northern blot (Clontech) was used. PTN cDNA probes specific for the ORF (2) or 5' untranslated exon U1 (287

nt fragment) or HERV-derived exon UV3 (257 nt fragment) were hybridized, washed and autoradiographed for 48 hours as described in Fang, et al. After exposure, blots were stripped and reprobed. GAPDH was used as a loading control.

5 **Insertion of Retroviral Elements into the Human PTN Gene.**
To elucidate the mechanisms that regulate expression of the human PTN gene, the 5' regions of mRNAs isolated from placenta and adult brain by 5' RACE PCR were examined. In particular, PTN expressed in placental tissues appeared of interest, since 10 placenta is mostly derived from fetal tissues and a human cDNA clone was originally reported from the screening of a placental library (See: Li, Y. S., Milner, P. G., Chauhan, A. K., Watson, M. A., Hoffman, R. M., Kodner, C. M., Milbrandt, J. & Deuel, T. F. (1990) Science **250**, 1690-1694). Furthermore, in situ 15 hybridization (See: Vanderwinden, J. M., Mailleux, P., Schiffmann, S. N. & Vanderhaeghen, J. J. (1992) Anat Embryol (Berl) **186**, 387-406) as well as Northern analysis with rodent trophoblast tissues had failed to detect a signal for PTN in contrast to a strong signal in Northern blots with human 20 placenta. Surprisingly, ten of eleven 5' RACE PCR clones with mRNA from placenta contained novel 5' UTR that are distinct from the previously described 5' UTR in human placental and brain cDNAs.

25 Sequence comparisons revealed that the novel 5' exons contained in the PTN mRNA from placenta are highly homologous to different regions of human endogenous retrovirus (HERV) type C. Analysis of human genomic DNA revealed that the HERV fragment is inserted in sense orientation into the intron region immediately upstream of the ORF of the human PTN gene 30 expanding this region relative to the murine gene. Low-stringency Southern blot analysis confirmed insertion of HERV also in the rhesus monkey genome and showed the lack thereof in murine genomic DNA. The most 5' HERV-derived PTN exon (UV3) is homologous to the viral 5' long-terminal repeat (LTR) region 35 and the downstream UV2 and UV1 exons are homologous to regions of the HERV gag, pol, and env pseudogenes (70, 85, and 80% identity respectively. (See: Repaske, R., Steele, P. E.,

O'Neill, R. R., Rabson, A. B & Martin, M. A. (1985) J Virol. **54**, 764-772; and Tomita, N., Horii, A., Doi, S., Yikouchi, H., Ogawa, M., Mori, T. & Matsubara, K. (1990) Biochem. Biophys. Res. Comm. **166**, 1-10.) Unlike infectious C-type viruses encountered today, all of which contain a tRNA^{Pro} primer binding site in their DNA (See Repaske, cited above), this prehistoric virus contains a tRNA^{Glu} primer binding site as its signature.

Expression of HERV-PTN Fusion Transcripts and of PTN Protein. The three HERV-derived exons are directly spliced to the first exon in the ORF of the PTN gene (O1) using splice donor sites distinct from the site reported in the retrovirus. The HERV-PTN fusion transcripts were present in placental cDNA at a ratio of 8:1:1 for UV3, UV2, and UV1, respectively, based on the number of clones obtained from the 5' RACE PCR analysis. Exon U1 spliced to O1 was found in only one of eleven placental 5' RACE PCR clones. This predominant expression of the HERV-derived exons in placenta contrasted with the lack of expression of these exons in brain; all seven 5' RACE PCR clones obtained from brain contained only exon U1 directly spliced to O1. Northern analysis with exon-specific probes confirmed this striking difference between placenta and brain. Other retroviral transcripts reported from placental tissues did not show cross-hybridization signals.

The HERV-PTN fusion transcripts were detected not only in human term placenta tissue (n=5) but also in the trophoblast of a fetus stillborn after 15 weeks of gestation, in primary cultures of cells grown from trophoblast biopsies obtained for prenatal diagnostics (9 to 12 weeks of gestation; n=7) as well as in trophoblast-derived human choriocarcinoma cell lines. In addition to Northern analysis, RNase protection studies and reverse transcription-PCR confirmed the presence of HERV-PTN fusion transcripts and the lack of U1 exon usage in JEG-3 and JAR choriocarcinoma cells. In contrast to the use of HERV-derived 5' exons in the trophoblast and choriocarcinoma, PTN mRNA from other embryonic or adult tissues and tumor cells contained U1 as their first 5' UTR exon, implying that these

transcripts originate from the promoter upstream of U1.

Western blot analysis showed that the PTN protein is produced and released into the culture medium by choriocarcinoma cells. This protein was mitogenically active and stimulated 5 colony formation of an indicator cell line, SW-13 cells.

Promoter Function due to the HERV Insertion. Primer extension with primers targeted within the most 5' UV3 exon of the HERV insert mapped the start site of the HERV-PTN transcripts to an A residue 39 nt downstream of putative CAAT and TATA boxes. Presence of these elements at the transcription 10 start site suggested that the HERV insertion might have generated an additional promoter in the intron immediately upstream of the coding region of the human PTN gene. To 15 investigate whether this putative promoter was responsible for the trophoblast-specific expression of HERV-PTN, transient transfection assays were performed with promoter-reporter constructs. Upstream of a luciferase reporter gene, a genomic fragment that starts 1.5 kb upstream of the transcription start 20 site that contains the CAAT and TATA boxes, and extends downstream of the start site into exon UV2 was inserted. Transcriptional activity of the resulting construct was observed exclusively in human choriocarcinoma cells (JEG-3 and JAR 25 cells), and only when the HERV insert was oriented as in vivo. Luciferase activity in JEG-3 and JAR cells transfected with the HERV promoter-reporter construct in sense orientation was 50- to 100-fold that of cells transfected with the promoterless vector alone. Deletion of the Alu element did not affect this 30 transcriptional activity. Only background activity was detected in PTN-positive human melanoma (1205LU) or in PTN-negative adrenal carcinoma (SW-13) cells. Therefore, it was concluded from these data that the HERV insertion in the human PTN gene generates a functional promoter that confers high tissue-specific expression.

Depletion of HERV-PTN with Ribozymes to Evaluate its 35 Biological Role. The biological significance of the expression of HERV-PTN in trophoblast-derived tissues was investigated using JEG-3 choriocarcinoma cells as a model system. Tumor

growth of these cells in experimental animals mimics the highly invasive and angiogenic growth phenotype of the normal human trophoblast as well as clinical choriocarcinoma. It was hypothesized that one of the contributing factors to this phenotype could be the expression of HERV-PTN. To address this hypothesis, the effects of reducing the abundance of HERV-PTN transcripts was studied in JEG-3 cells by stable expression of a PTN-targeted ribozyme. A vector (pTET/Rz261) with high transcriptional activity in these cells was used to express the ribozyme.

5 Northern blot analysis revealed that ribozyme expression reduced the amount of HERV-PTN mRNA in JEG-3 cells to background levels. No difference in the proliferation rate of PTN-depleted versus control cells was apparent in vitro, suggesting 10 that the cells do not require PTN as an autocrine growth factor even though they secrete the protein in a biologically active form. However, a marked difference in the growth phenotype of PTN-depleted versus control cells was observed after xenografting tumor cells into athymic nude mice. In an initial 15 study, the tumor cells were implanted into their "natural", intra-abdominal environment to observe their orthotopic growth behavior. The control cells formed large tumor masses that invaded the abdominal organs within 2 to 3 weeks, whereas only 20 a few, small seedings of PTN-depleted tumor cells were detected in the abdomen at the end of the study (n=5 and n=4 animals 25 respectively). Parallel results were obtained after subcutaneous injection of tumor cells. In contrast to control cells, which grew rapidly into highly angiogenic tumors (n=7). No tumor growth was observed with PTN-depleted cells (n=9). These 30 observations indicate that PTN is an essential and rate-limiting factor for choriocarcinoma growth, invasion, and angiogenesis in vivo.

35 This invention shows that integration of an HERV element into the human PTN gene generates a novel tissue-specific promoter not present in the common ancestral gene from which the primate and murine PTN genes descended. Transcriptional activity of this promoter results in the expression of HERV-PTN

fusion transcripts specifically in human trophoblast-derived normal and tumor cells. The integration site of the HERV fragment shows several features indicative of inserted retroelements, i.e. location at the 5' region of a gene (See: 5 Rohdewohld, H., Weiher, H., Reik, W., Jaenisch, R. & Breindl, M. (1987) J. Virol. **61**, 336-343), presence of an Alu sequence of 280 nucleotides and of a stretch of 26 A residues at its 5' end.

10 The HERV found in the human PTN gene represents a non-infective, replication-defective prototype of a retrovirus that integrated into ancestral DNA before the divergence of apes (including human predecessors) and Old World monkeys over 25 million years ago. It is quite surprising that altered expression of human genes due to the insertion of retroviral 15 elements appears to be an extremely rare event, bearing in mind the high number of HERV fragments found integrated throughout the human genome (several thousand copies). On the other hand, an alteration of a gene expression pattern will only penetrate 20 during phylogenesis, if a better survival chance is associated. Functional studies now show that ribozyme-mediated depletion 25 of HERV-PTN mRNA in human choriocarcinoma cells reverses their highly aggressive growth phenotype in an in vivo model. The superficial, less invasive implantation of the murine trophoblast coincides with the lack of trophoblast-specific PTN gene expression.

30 The promotor of the invention is particularly useful for testing effectiveness of agents for suppression of growth and invasiveness of malignancies of trophoblastic origin by making it possible to grow highly aggressive choriocarcinoma cells, then exposing the tissue culture to the agents being studied for their expression. By the use of the cells containing the vector, it is possible to do extensive screening of possible agents for use in treating such malignancies.

35 The ribozyme of the formula:

'5-GTTGCAGGGCTGATGAGTCCGTTAGGACGAAATCTTACATC-3'

may be administered to treat or prevent human choriocarcinoma growth, invasion and angiogenesis in a pharmaceutically

acceptable carrier alone or in a vector such as pTET/Rz261. The ribozyme may be administered by any method which causes the ribozyme to contact and enter the cell. For example, compositions containing 0.1 to 100 μ g/ml of the sequence may be prepared in solutions such as buffered saline, half-normal saline, glucose in saline, etc. The sequence may be administered, for example, in liposomes. For example, a composition containing 5 μ g/ml of the sequence in saline may be infused into the uterus following removal of a hydatidiform mole. The dosage administered will depend on the size and condition of the patient and on the tissue targeted.

The sequences reported for the herein have been deposited in the GenBank data base and assigned accession number U71455 and U71456.

What we claim is:

5 1. A method of inhibiting growth, invasion and angiogenesis of a tumor containing human endogenous retrovirus-pleiotrophin (HERV-PTN) sequences comprising administration of a choriocarcinoma inhibiting amount of PTN-targeted ribozymes.

10 2. A method of inhibiting growth, invasion and angiogenesis of a tumor containing human endogenous retrovirus-pleiotrophin (HERV-PTN) sequences comprising administration of a choriocarcinoma inhibiting amount of a ribozyme of the sequence:

15 '5-GTTGCAGGGCTGATGAGTCCGTTAGGACGAAATCTTACATC-3'

20 3. A method of claim 2 wherein the ribozymes are administered in a vector containing pTET/Rz261.

25 4. A composition of matter comprising a sequence of the formula:

20 '5-GTTGCAGGGCTGATGAGTCCGTTAGGACGAAATCTTACATC-3'
in a pharmaceutically acceptable carrier.

25 5. A composition of claim 4 wherein the sequence is delivered in a vector.

30 6. A composition of claim 4 containing 0.1 to 100 µg/ml of the sequence:

'5-GTTGCAGGGCTGATGAGTCCGTTAGGACGAAATCTTACATC-3'.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/21959

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/70

US CL : 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/ 91.31, 172.3, 320.1; 514/44; 536/24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN (BIOSIS, CANCERLIT, LIFESCI, MEDLINE, INPADOC, WPIDS).

Search terms: pleiotrophin(s), choriocarcinoma, tumor, retrovirus, ribozyme(s).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	SCHULTE et al. Human Trophoblast and Choriocarcinoma Expression of the Growth Factor Pleiotrophin Attributable to Germ-Line Insertion of an Endogenous Retrovirus. Proceedings of the National Academy of Sciences USA. 10 December 1996, Vol. 93, No. 25, pages 14759-14764, see entire document.	1
X	CZUBAYKO et al. Ribozyme-Targeting Elucidates a Direct Role of Pleiotrophin in Tumor Growth. The Journal of Biological Chemistry. 19 August 1994, Vol. 269, No. 33, pages 21358-21363, see entire document.	1

Further documents are listed in the continuation of Box C. See patent family annex.

• Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

04 FEBRUARY 1998

Date of mailing of the international search report

25 FEB 1998

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INTERNATIONAL SEARCH REPORT

International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2. Claims Nos.: 2-6 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
the claims are drawn to a method of using specific nucleic acid sequences and require a search of said nucleic acid sequence. However, a sequencing listing in computer readable format has not been provided, preventing a search of the claimed subject matter.
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.